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# AN EVALUATION OF THE PHOTOPIC ELECTRORETINOGRAM USING LOWER EYELID ELECTRODES

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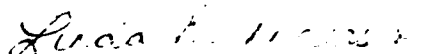Brooks Air Force Base, TX 78235-5301

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NOTICES

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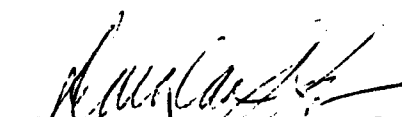
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The voluntary fully informed consent of the subjects used in this research was obtained in accordance with AFR 169-6.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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The use of corneal contact lens electrodes for recording Electroretinograms (ERG) is difficult in some patients (children) and impossible in others (hyperbaric patients) due to unusual recording conditions. After reviewing the various published methods already tested, we implemented a technique that employs a lower eyelid electrode. In addition, a "Ping-Pong ball Ganzfeld" was fabricated to mimic the Ganzfeld dome typically used in clinical laboratories. Three questions were then addressed: (1) the adequacy of the method for acquiring photopic ERGs; (2) the influence of the direction of gaze on ERG parameters; (3) the day-to-day reliability of the method. When ERGs derived from contact lens electrodes were compared with those obtained from lower eyelid electrodes under the same test conditions, it was found that the lower eyelid electrode waveforms were smaller in amplitude but identical in shape to waveforms produced by corneal contact lens electrodes. The influence of gaze direction on ERG parameters was tested while the subjects fixed their gaze to the right, to the left, and up and down at 5° intervals from 0° - 30°. The position of gaze did not

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). ABSTRACT (Continued)

influence ERG values for any of the test conditions. In order to establish the reliability of the method, ERGs from one subject were recorded on six different days over a period of four months. The lower eyelid electrode proved to be an excellent ERG recording method, especially under difficult test conditions.

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# AN EVALUATION OF THE PHOTOPIC ELECTRORETINOGRAM
## USING LOWER EYELID ELECTRODES

## INTRODUCTION

Children and patients with corneal disease are not good candidates for corneal contact lens electrode (CCLE) wear. Since electroretinography was first practiced, there has been a need for a good electroretinographic (ERG) recording method that avoids the use of contact lens electrodes. Other unusual recording conditions also preclude the use of corneal electrodes. Some ophthalmology patients undergoing hyperbaric oxygen treatment must have their ERGs monitored repeatedly. The hyperbaric treatment protocol involves a 3-hr session, repeated twice a day for 10 days. Corneal contact lens electrode use, under these conditions, is impossible because of the increased discomfort associated with long-term wear and the increased chance of corneal abrasion. Repeated corneal anesthetic application is not advisable. Faced with these conditions and the need for ERG recordings, we implemented a method of ERG testing that closely approximates clinical ERG conditions.

The properties of some dermal electrodes for ERG recording and related electrode placements have been studied extensively(1-8). Nakamura(1) recorded ERGs from fifteen different electrode placements on the face. He found that locating the active electrode on the lower eyelid produced the largest amplitudes with the fewest artifacts. Other investigators have experimented with different electrode placements, such as the inner and outer canthi(2,3) and supraorbital and nasal loci(4). They found that the placement of the reference electrode does not appear to be as critical and agreed that the lower eyelid was the most suitable site for the active electrode. A basal electrode of the McLean type(4), the ear(1,2), and the outer canthus(5) have all been used successfully for reference electrodes, while using an active lower eyelid electrode.

Other studies(1,3) have investigated the effect of gaze direction on the ERG when using dermal electrodes. Noonan et al.(3) recorded ERGs from subjects while their gaze was at the primary gaze position and 35° upward, downward, lateral, and medial to the primary visual axis. For each gaze position, the stroboscopic photostimulator was moved, so that its axis coincided with the subjects' visual axis. He recorded from four periorbital locations: the bridge of the nose; external canthus; infraorbital ridge; and above the eyebrow. In comparing

electrode responses, he found that the ERG reached maximum amplitude when the cornea was nearest that electrode. Nakamura(1) investigated the same four periorbital electrode placements. However, with his subjects, the gaze was fixed on the center of the stimulating system while the head was turned to cause abduction, adduction, supraduction, and infraduction. He also found that the ERG amplitudes became larger as the distance between the cornea and the recording electrode decreased. Further, he determined that recording from the lower eyelid electrode was least affected by deviation of the eye, and that the lower eyelid location produced the greatest ERG amplitudes.

For the above-mentioned reasons, and due to our own observations, the lower eyelid electrode (LELE) location was selected for the evaluation of patients undergoing hyperbaric oxygen treatment. Our experimental setup was unique, however, in that we used the dermal electrode method with a Ping-Pong ball Ganzfeld in order to simulate a standard clinical protocol using a Ganzfeld dome. Since the Ganzfeld removes accommodation and fixation cues, the eyes may move to an unknown position. The influence of gaze position on the parameters of the ERG measurements was unknown for our unique recording situation, and, therefore, further analysis was required.

Because of the frequency of hyperbaric treatment (twice daily for up to 10 days), the day-to-day variability of the ERG waveform may be a critical variable in determining the efficacy of treatment. Karpe(9) determined that, when a CCLE was used, intrasubject ERG variation was no more than 10% over a period of 11 months. Giltrow-Tyler et al.(2) repeated tests with 8 subjects and found high repeatability (r=.98 p<.01) after 6 weeks, when retesting with lower eyelid electrodes, suggesting that little or no individual day-to-day variability occurs. However, no studies have evaluated responses obtained with the combination of lower eyelid electrodes and a "Ping-Pong ball Ganzfeld" stimulator as used in our protocol.

## MATERIALS AND METHODS

An attempt was made to model closely the clinical photopic ERG test protocol currently used in the Electrophysiology Laboratory at the USAF School of Aerospace Medicine (USAFSAM). The patients tested under this standard protocol have a reference electrode taped above the eyebrow of each eye, an active corneal contact lens electrode, and an earclip ground. The seated patient's eyes are dilated; the chin is on a chin rest at the opening of a Ganzfeld dome. This clinical protocol was imitated for the hyperbaric patients, using a lower eyelid skin electrode rather than a corneal contact lens electrode; the Ping-Pong balls simulated the Ganzfeld(10).

2

## Electrodes and Patient Preparation

The right eye of each subject was dilated with 1% Tropicamide and 2.5% Phenylephrine HCl Ophthalmic solution. The area above the eyebrows, the lower eyelids, and ear were scrubbed with a 10% Benzalkonium Cl solution and rubbed with a small amount of electrode paste prior to electrode placement. A 16 mm silver-silver chloride Beckman biopotential reference electrode was attached to the temporal area (above each eyebrow) with an adhesive collar. An 11 mm Beckman biopotential electrode was similarly taped to the lower eyelid to become the active electrode. An earclip electrode was attached to the ear to establish an electrical ground. The electrodes were stored in 0.9% saline and rinsed briefly before use, to minimize battery effects.

## Stimulus

A 150W flood light was used as an adapting light. A variable transformer (Variac) was used to permit daily adjustment of the light's intensity. A calibrated Tektronix J16 photometer was used to set the intensity before each experiment. The light was adjusted until the photometer read 30 $\pm$0.5 fL. A Grass PS2 photostimulator, set at intensity 16, was used to produce the flash stimulus with a repetition rate of 1 flash/sec. Both the adapting light and the flash lamp were placed 61 cm (24 in.) from the subjects' eyes. Inside the hyperbaric chamber, the lights can be placed no closer to the patient than 61 cm (24 in.).

## Test Configuration

Six subjects were tested. Informed consent was obtained from each subject, after the details of the study had been explained. The subject wore a pair of specially fabricated "glasses". The right eye was covered with half of a Ping-Pong ball which functioned as a Ganzfeld. The left eye was covered with a 0.5 log unit neutral density filter to allow the subject to gaze in the required direction without experiencing discomfort from the adapting light. The positions for gaze direction were marked with a red light-emitting diode (LED) positioned on a steel rod that served as a perimeter. The perimeter was indexed at $5^\circ$ intervals beginning with $0^\circ$ and ending with $30^\circ$. The perimeter could be pivoted to permit calibrated right, left, up, and down fixation light positions. The seated subject's chin and forehead were steadied by a headrest to maintain the head in the primary position. The subject's gaze was fixed on the LED as it was moved from $0^\circ$ to $5^\circ$, $10^\circ$, $15^\circ$, $20^\circ$, $25^\circ$, and $30^\circ$ for each direction (right, left, up, and down). We consider it unlikely that anyone's gaze would be fixed at positions more extreme than $30^\circ$ from the primary

position, since those positions are uncomfortable and difficult to maintain.

One of the 6 subjects was tested on 6 different days over a 4-month period. The test methods and parameters, as described above, were used with the exception of gaze deviation. Recording the ERG with a change of gaze direction every $5^{\circ}$ was not necessary to answer questions about day-to-day response variability. Increments of $15^{\circ}$ in each gaze direction around the primary position were considered sufficient. The subject's gaze was fixed on the LED during recording, and the LED was moved to $0^{\circ}$, $15^{\circ}$, and $30^{\circ}$ for right, left, up, and down directions.

## Recording Conditions

A PDP 11/44 computer was used to signal average the flash responses. The responses were amplified 2000 times with Grass Instrument Company P511 biological amplifiers. High and low band pass filters were set at 1 kHz and .3 Hz respectively. An artifact rejection algorithm was incorporated in the computer program to reduce artifacts created by blinks and other muscle contractions. For most subjects, a threshold level of $100 \mu V$ was adequate to satisfactorily reduce contamination from artifacts. Thirty-two flash responses were averaged at each gaze position. A 160-msec time base represented 256 data points. Other computer programs plotted graphs of the waveforms and determined the negative and positive peaks of the data. The peaks were independently validated by two investigators to ensure data accuracy.

## RESULTS

Lower eyelid electrodes produce ERG waveform records identical to those obtained using CCLEs. As shown in Figure 1, a negative A-wave and a larger positive B-wave are recorded and, although the amplitudes are smaller, the latencies are within normal limits(10). When compared to the CCLE recordings, the amplitudes from the LELE recordings are reduced by approximately 80%. That result is in good agreement with data from a previous study(6). Furthermore, the features of the "Ping-Pong ball Ganzfeld" ERGs compare well with dome Ganzfeld ERGs.

The data were partitioned, so that a two-way analysis of variance (ANOVA) could be performed to assess the influence of gaze on ERG parameters. A power test was run and 6 subjects were found to be sufficient. The grand means, standard deviations, range and the F-ratios for A-wave latencies, A-wave amplitudes, B-wave latencies and B-wave amplitudes, for all gaze conditions combined, are listed in Table 1. The ANOVA was non-significant across gaze direction ($P \geq .4991$), demonstrating
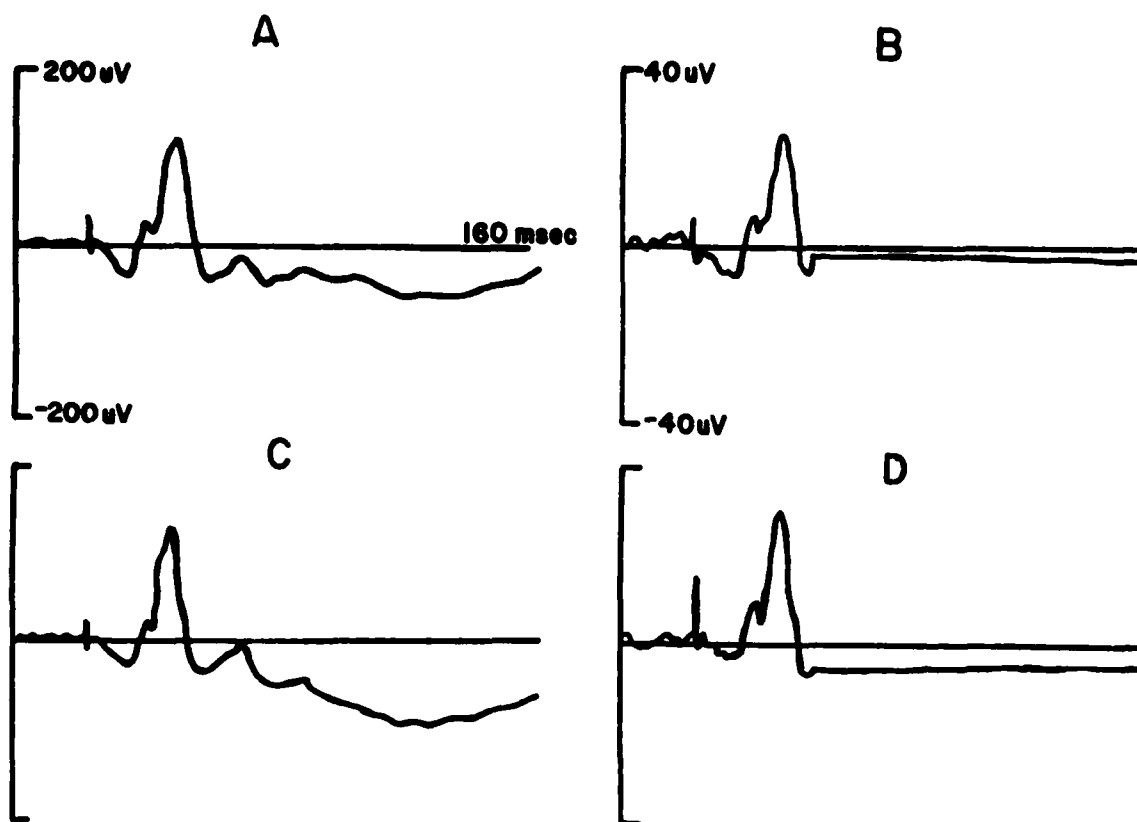
**Fig 1.** Comparison of ERGs recorded with corneal contact lens ERG electrodes (CCLE) and lower eyelid ERG electrodes (LELE). Tracings A and B compare CCLE and LELE waveforms in a Ganzfeld dome. Tracings C and D compare CCLE and LELE waveforms using a Ping - Pong ball Ganzfeld.

**TABLE 1.** MEANS, RANGES, STANDARD DEVIATIONS (SD) AND F TEST VALUES FOR A-WAVE AND B-WAVE AMPLITUDES AND LATENCIES.

| WAVE | AMPLITUDES($\mu$V) | | | LATENCIES(msec) | | |
|------|-------|-------------|------|-----------|-------------|-----|
|      | RANGE | MEAN (F)    | SD   | RANGE     | MEAN (F)    | SD  |
| A    | 2.8-4.2 | 3.6 (.99) | .29  | 13.0-15.0 | 14.1 (.49)  | .61 |
| B    | 15.6-19.5 | 17.9 (1.00) | 1.09 | 27.3-28.2 | 27.8 (1.00) | .26 |

5

TABLE 2.   MEANS AND STANDARD DEVIATIONS (SD) OF THE A-WAVE AND
          B-WAVE AMPLITUDES AS A FUNCTION OF GAZE DIRECTION.

| DIRECTION | A-wave AMPLITUDES($\mu$V) | | B-wave AMPLITUDES($\mu$V) | |
|---|---|---|---|---|
| | MEANS | SD | MEANS | SD |
| right | 3.73 | .17 | 18.4 | .70 |
| left | 3.46 | .32 | 17.6 | .93 |
| up | 3.36 | .31 | 18.4 | .81 |
| down | 3.72 | .20 | 17.6 | 1.39 |

that these data were derived from the same underlying
population.  Means and standard deviations for the A-wave and
B-wave amplitudes, as a function of gaze direction, are shown in
Table 2.  There is less than a 10% difference between means for
these conditions.

     The means and standard deviations of the A-wave and B-wave
latencies and amplitudes, as a function of the 6 recording days,
are listed in Table 3.  These data were partitioned, so that the
influence of day-to-day variability could be evaluated.  A
one-way ANOVA revealed that there were no significant day-to-day
influences on the A-wave latencies and amplitudes, nor on the
B-wave latencies. The B-wave amplitudes, however, were
significantly different ($\alpha$=.05).

TABLE 3.   MEANS AND STANDARD DEVIATIONS OF A-WAVE AND B-WAVE
           AMPLITUDES AND LATENCIES, FOR ALL RECORDING
           CONDITIONS COMBINED (12 CONDITIONS), AS A FUNCTION
           OF RECORDING DAY.

| DAY | AMPLITUDES($\mu$V) | | LATENCIES(msec) | |
|---|---|---|---|---|
| | A-wave (SD) | B-wave (SD) | A-wave (SD) | B-wave (SD) |
| 1 | 2.7 (.35) | 10.5 (1.9) | 14.6 (1.3) | 28.9 (.93) |
| 2 | 2.9 (.60) | 10.2 (1.4) | 14.6 (1.9) | 29.1 (1.04) |
| 3 | 3.2 (.90) | 11.4 (2.1) | 14.5 (1.1) | 28.9 (.95) |
| 4 | 3.2 (1.1) | 10.6 (2.2) | 13.3 (2.8) | 28.9 (1.49) |
| 5 | 3.0 (.82) | 11.7 (1.5) | 13.1 (1.9) | 29.7 (1.33) |
| 6 | 2.8 (.46) | 12.2 (1.5)* | 13.5 (1.8) | 29.1 (1.13) |

* indicates a significant difference from the other 5 days
  (one-way Anova and Duncan's multiple range test ($\alpha$=.05)).

A Duncan's multiple range test was employed to further analyze
the data and showed that the B-wave amplitudes collected on the
6th day of recording were significantly different from the other
values. There was a 16% difference in B-wave amplitudes for the
6th recording day with respect to the smallest recorded value.


## DISCUSSION


The results obtained in this study demonstrate that lower
eyelid ERG electrodes produce ERGs that are comparable in shape
and latency to those recorded with corneal contact lens
electrodes. The latencies found, when using the lower eyelid
electrode, were in agreement with Berry's(5) results and were
well within the accepted range of values for normal subjects
whose ERGs were recorded with contact lens electrodes(10).

ERG amplitudes in this study were somewhat lower than those
previously reported, especially for the A-wave. In previous
studies, amplitudes have ranged from $5\mu V$ – $18\mu V(7)$ for the
A-wave and $5\mu V(7)$ – $62.7\mu V(2)$ for the B-wave. This result is
not surprising, since the testing conditions were dissimilar.
The variability is, no doubt, due to differences in stimulus and
testing parameters. For example, Nakamura(1) used an undilated
eye with a Ganzfeld background luminance of 5 mL and an
orange-red test stimulus. Although he does not report the
recorded amplitudes, careful measurement of the published
waveforms reveals A- and B-wave amplitudes of approximately
$8.3\mu V$ and $20.6\mu V$, respectively. In another study, Noonan et
al.(3) used a dilated eye, a background luminance of between 2.6
and 3.0fL, and ambient room light of 8 fL with direct flash tube
stimulation. During "central gaze", he recorded $16.3\mu V$ A-wave
amplitudes and $24.0\mu V$ B-wave amplitudes. The mean amplitudes
for the A- and B-waves reported here were $3.6\mu V$ and $17.9\mu V$.

Although our results are similar to Nakamura's, there are
obvious differences. It is known that background luminance,
flash intensity, amount of mydriasis, and use of a Ganzfeld
(versus direct flash) all have an effect on ERG data(10). For
instance, as the background luminances increase, there are
concomitant increases in ERG amplitudes, until a threshold is
reached. At that point, amplitudes decrease with increasing
background luminances, due to the desensitization of the
cones(10). To provide conditions similar to those used
clinically, it was necessary to use a 30-fL background
luminance, which is greater than others have reported using,
when recording from eyelid electrodes. That luminance level
undoubtedly accounts for the smaller amplitudes recorded in this
study.

Noonan et al.(3) and Nakamura(1) showed an increase in ERG amplitudes as the cornea was moved closer to the active electrode (e.g., in downward gaze). This increase, however, was not found to be uniform in the present study. Some subjects in this study had ERG amplitudes that increased slightly with downward gaze, which is in agreement with Nakamura's(1) result. That observation was not the case for all subjects, as may be seen in Table 3. Again, this result may be due to differences in experimental parameters. Noonan et al.(3) found that, under all but one condition, the amplitudes of the A- and B-waves were greatest for an "inferior" electrode placement (lower eyelid).

Since the studies of Noonan et al.(3) and Nakamura(1) utilized stimulus geometrics (direct flash) different from ours, there is no simple way to compare our data with theirs. We must assume that test condition differences have produced the observed differences.

Individual daily variability in ERG waveform recordings(11), as shown in Table 3, are in agreement with those previously reported. Karpe(9) found an average amplitude difference of 6%, with respect to the smallest mean amplitude value (with a maximum difference of 15%), when comparing intrasubject ERGs recorded periodically during an 11-month period. These data have a statistically significant 16% difference in B-wave amplitudes for the 6th recording day, with respect to the smallest recorded value (Day 2). That result agrees well with the 15% difference reported by Karpe(9). Finkelstein and Gouras(11) have stated that there should be no more than a 10% difference in daily ERG amplitudes, when recording from the same subject. Although a statistically significant difference was found between the 2nd recording day (smallest mean amplitude value) and the 6th recording day (largest mean amplitude value), the $12.2 \mu V$ amplitude of the 6th recording day B-wave is only 4% different from the $11.7 \mu V$ amplitude recorded on the 5th day. These observations are in good agreement with those of previous authors(7,9,11).

A multitude of variables have been reported to influence the ERG. Nicotine, hypertension(12), and hyperventilation(13) have been shown to effect an increase in B-wave amplitudes. The rapid intake of alcohol(14) can decrease the B-wave amplitude. Watanabe et al.(15) found decreases in the A- and B-wave amplitudes both during and after exercise. Diurnal(16) and circadian(17) ERG rhythms have been shown to exist. Birch et al.(16) concluded that 2 days of entrainment, with a light-dark cycle consisting of 14 hours of light and 10 hours of darkness, maximized the diurnal rhythm in the rod ERG. Nozaki et al.(17) showed the ERG B-wave to be significantly larger at noon, suggesting the presence of a circadian rhythm. Strict control of such variables is difficult and was not possible in this study. Any one of them could have resulted in the single significant difference in B-wave amplitudes.

The lower eyelid dermal electrode, as demonstrated in this study and others, has proven to be more than adequate for collecting ERG data. It may be utilized conveniently in "hostile" environments and when corneal contact lens electrodes are unsuitable. Our original intent was to develop a method of recording ERGs that would allow the investigator to determine changes in the ERG due to oxygenation under pressure. Noncorneal electrodes placed on the lower eyelid proved to be an effective means of collecting ERG data. A larger·study is being conducted to establish norms under standardized field testing conditions, before this method is used as a clinical testing procedure(18).

## ACKNOWLEDGMENTS

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